Biomedicine & Pharmacotherapy 106 (2018) 54–60

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

The intervention effect of zuogui pill on chronic kidney disease-mineral and bone disorder regulatory factor

Xiaohong Ma[a,*], Liqun He[b]

[a] Chenxinghai Hospital of Zhongshan City, No. 18 Zhuyuan Road, Xiaolan Town, Zhongshan, Guangdong, 528415, China
[b] Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine and Pharmacology, No. 185 Pu'an Road, Huangpu District, Shanghai, 200021, China

ARTICLE INFO

Keywords:
Zuogui pill
Chronic kidney disease
Mineral and bone disorder
Bone metabolism
Nourish kidney

ABSTRACT

Chronic kidney disease-mineral and bone disorder (CKD-MBD) play a critical role in the pathogenesis of cardiovascular complications in patients with chronic kidney disease (CKD). Zuogui pill as a traditional Chinese herbal drug has been used for nourish kidney essence improve bone malnutrition of renal bone disease by regulating the metabolism of calcium and phosphorus and participating in osteoblast metabolism. In the present study, 5/6 nephrectomy rat model was used to reveal the mechanism of zuogui pill in treatment of CKD-MBD. Compared with sham rats, the levels of serum phosphorus, PTH, iPTH and creatinine were significantly decreased, while the serum calcium level was significantly increased, and the Cbfa1 protein level was significantly decreased and FGF23 protein level was significantly increased by Zuogui pill treatment. Compared with model rats, the BMD of rat was significantly increased by Zuogui pill treatment. Histological analysis revealed that the kidney injury of rats with CKD was significantly reduced by zuogui pill treatment. Compared with model rats, the CYP27B1 mRNA level was significantly increased, and the PTH mRNA level and NaPiIIa protein level were significantly decreased in the kidney by zuogui pill treatment. We inferred that zuogui pill exhibited potential therapeutic effects on CKD-MBD in the rats by regulating bone metabolism and nourish kidney.

1. Introduction

Chronic kidney disease (CKD) with morbidity and mortality is a worldwide public health problem. Progressive tubulointerstitial fibrosis is the final common pathway for all kidney diseases leading to chronic renal failure [1–3]. The histopathology of tubulointerstitial fibrosis features deposition of interstitial matrix in association with activation of renin-angiotensin system, inflammation and oxidative stress, TGF-β/Samd and lipid metabolism [4–9]. Chronic kidney disease-mineral and bone disorder (CKD-MBD) is one of the serious complications occurring in the patients CKD. It is manifests as metabolic abnormalities in calcium, phosphorus, parathyroid hormone (PTH) and vitamin D, and bone abnormalities and extraskeletal calcification [10]. FGF23 as a novel factor adjustable for phosphorus, can reduced the blood phosphorus level by down-regulating the expression of NaPiIIa and CYP27B1 in kidney and involving the development of vascular calcifications in CKD [11,12]. The bone abnormalities of CKD-MBD include renal osteodystrophy, the disorder of mineral metabolism and volume, and increased propensity to fracture [13]. Recently, vitamin D agents and calcium supplementation are frequently administered to control blood calcium of patients with CKD-MBD in the clinical setting. Moreover, vitamin D is known to have several pleiotropic effects on bone and mineral metabolism, immune function and cardiovascular systems [14]. However, the effects of those drugs are complicated and an excessive administration of drugs can lead to the induction of vascular calcification, poor efficacy of patients with advanced CKD [15]. Several guidelines on the treatment of CKD-MBD have been published but large gaps between guidelines and clinical practice still exist [16]. Consequently, it is necessary to clarify the mechanism of CKD-MBD and to achieve appropriate management strategies for CKD-MBD.

Zuogui pill as a traditional Chinese herbal drug has been used for nourish kidney essence. Several studies have been reported that zuogui pill induced the release of sex hormones and promoted recover from ovary damage [17,18]. Furthermore, zuogui pill can inhibit the apoptosis of nerve cells by improving differentiation and recovery in nerve cells, improve immunity and promote the restore of immune system balance [19,20]. In addition, zuogui pill can enhance the therapeutic effect of osteoporosis treatment and improve bone malnutrition of renal bone disease by regulating the metabolism of calcium and phosphorus and participating in osteoblast metabolism [21,22]. It was reported that

* Corresponding author.
E-mail address: 743457870@qq.com (X. Ma).

https://doi.org/10.1016/j.biopha.2018.06.092
Received 12 May 2018; Received in revised form 15 June 2018; Accepted 15 June 2018

the secretion of bone Gla-protein (BGP) in osteoblasts was affected zuogui pill in rat's serum [23]. Several studies have confirmed that zuogui pill exerted effective treatment for osteoporosis, but the intervention of renal bone disease was rarely reported. Our previous study was also revealed that zuogui pill can decrease the levels of alkaline phosphatase (ALP), intact parathyroid hormone (iPTH) and blood phosphorus in rats model with 5/6 subtotal nephrectomy [24]. However, the main mechanism of zuogui pill on treatment of CKD-MBD still needs further study.

The present study established a rat model of CKD-MBD and used that to investigate the effect of zuogui pill on improving renal bone disease. Calcitriol or herbal treatment are used as positive control [25,26]. The molecular mechanism of zuogui pill was explore by detect the protein levels of fibroblast growth factor 23 (FGF-23), CYP27B1 and NaPiIIa. In order to provide new ideas and guiding for clinical treatment of renal bone disease.

2. Materials and methods

2.1. Reagents

Zuogui pill was purchased from Shanghai Leys Pharmaceutical CO., LTD (Z31020371) and composed of *cooked rehmannia* root, *cornus officinalis*, *Chinese yam* rhizome, *Chinese dodder* seed, *Antler glue*, *Colla carapacis*, *Wolfberry fruit*, *Cyathula* root and *Poria cocos*. Herbal was composed of *Drynaria fortunei* 30 g, *Angelica sinensis* 30 g, *Rehmannia glutinosa* 30 g, *epimedium* 30 g, *Astragalus membranaceus* 30 g and *Rheum officinale* 12 g, all herbals were purchased from Sanjiu Medical & Pharmaceutical CO., LTD. Calcitriol was purchased from MedChenExpress (Monmouth Junction, USA).

2.2. Experimental animals

Forty-eight Wistar rats (200 ± 20) g were obtained from Shanghai Sippr-BK laboratory animal Co. Ltd. Ten rats was fed as normal group and referred to hereafter as the control group. Forty rats were performed as CKD-MBD model by 5/6 nephrectomy [27]. At 8 weeks of age, the rats underwent 2/3 nephrectomy of the left kidney, followed by total nephrectomy of the right kidney one week later. After nephrectomy, the rats were fed with high phosphorus diet (1.0% calcium, 1.2% phosphate) and randomly assigned to the following four groups: (i) eight rats as model group, (ii) eight rats were treated with zuogui pill 0.64 g drugs/200 g body weight, once each day, for 12 weeks (zuogui pill group), (iii) eight rats were treated with calcitriol 0.009 μg drugs/200 g body weight (1/60 of the adult daily normal oral dose) [28], (iv) eight rats were treated with herbal 5.83 g drugs/200 g body weight. Eight rats were performed same operate but not resection of kidney. Eight normal rats were performed as control group.

2.3. Blood samples collection and processing

Rats tail vein blood samples were collected and used to measure serum calcium (Ca), phosphorus (Pi), PTH, iPTH and creatinine (Cr) levels. Serum samples were collected following centrifugation for 5 min at 1000*g*. Blood calcium, phosphorus and creatinine levels were measured by Fuji DriChem 3500 system (Tokyo, Japan). Serum PTH and iPTH levels were detected using Rat PTH-ELISA kit (cat. No. 69-30397, mskbio) or Rat iPTH-ELISA kit (cat. No. 60-2500) (immutopics, San Clemente, USA).

2.4. Bone mineral density (BMD) detection

After 12 weeks of intervention, all the rats per group were anesthetized with 50 mg/kg ketamine, and the femur form each animal were scanned using dual-energy X-ray absorptiometry (GE, USA).

2.5. Hematoxylin and eosin (H&E) and Masson staining

H&E and Masson staining were performed as previously described [29,30]. Briefly, the kidneys were fixed in 10% formalin. After fixation, issue sections were dehydrated with graded ethanol and embedded in paraffin. Sections of 5 μm of paraffin-embedded tissues were mounted on glass slides, rehydrated with distilled water and the sections were used for H&E and Masson staining. The samples were observed using light microscopy.

2.6. Periodic acid schiff (PAS) staining

Tissue samples were fixed with 95% alcohol for 10 min, prior to being washed, dried, stained by 1% periodic acid for 20 min and washed and dried once more. Each sample was then stained by a schiff reagent for 60 min, washed and dried, and subsequently stained by hematoxylin for 5 min, washed and dried. The samples were observed using light microscopy.

2.7. Quantitative real-time PCR

Total RNA was extracted from all samples with Trizol (Takara, Dalian, China) and detected by an ultraviolet spectropho-tometer and agarose electrophoresis. Quantitative real-time PCR was performed as previously described [31]. 1 μg RNA was reverse transcribed to obtain first-strand cDNA using the PrimeScript®RT reagent Kit with gDNA Eraser (TaKaRa, Dalian, China) according to manufacturer's instructions. Expression patterns of all target genes CYP27B1, BGP and PTH were analyzed using quantitative real-time PCR (qRT-PCR). Primer premier 5.0 was used to design the fluorescent primers before gene synthesis performed. The synopsis of primers for each gene was listed in Table 1. Primer efficiency was determined by performing serial dilutions of reference cDNA. Reaction (20 μl total volume) contained 10 μl of 2 × SYBR Premix Ex TaqTM (TaKaRa, Dalian, China), 0.40 μmol/l each primer and 0.1 ± 0.02 μg of cDNA template. The following three-step qRT-PCR reaction was performed: pre-denaturation at 95 °C for 30 s, followed by 40 cycles of denaturation at 95 °C for 5 s, annealing at 58 °C for 20 s and elongation at 72 °C for 20 s. The transcriptional levels of genes were calculated using the ΔΔCt method [32]. The threshold cycle (Ct) was determined for each reaction, by using ΔΔCt method can make Ct values for each gene of interest were normalized to the endogenous control gene (GAPDH). For each group, three samples were measured and three technical replicates of each measurement were obtained.

2.8. Western blot analysis

For total protein extraction, the tissue was homogenizse in RIPA lysis buffer containing protease inhibitor at 4 °C. Both cell lysate and tissue lysate were centrifuged at 12,000 × *g* for 15 min and supernatants were collected. The protein concentration was determined by BCA kit (Bioswamp, China). Western blot analysis was performed as previously described [33]. Equal amounts of protein (30 μg) were separated by 10% SDS-polyacrylamide gels. After electrophoresis, proteins were transferred onto PVDF membrane (Millipore, USA) at 200 mA for 2 h. The membranes were blocked for 2 h at room temperature with 5% skim milk in Tris-buffered saline (20 mmol/l Tris,

Table 1
primers used for RT-qPCR analysis.

Gene	Forward primer (5'–3')	Reverse primer (5'–3')
CYP27B1	CGGGACACTTCGCACAGTT	TTCACCATCCGCCGTTAGC
PTH	GCACAACCTGGGCAAACAC	TTCCTCCTTCTTGGTGGGC
GAPDH	TGATTCTACCCACGGCAAGTT	TGATGGGTTTCCCATTGATGA